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Diagnostic ex vivo assay of glucose in live cell using voltammetry

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Abstract : The hand held voltammetry systems searched diabetic assay using glucose sensor of fluorine nafion doped carbon nanotube electrode (FCNE). An inexpensive graphite carbon pencil was used as an Ag/AgCl reference and Pt counter electrode. Upon combining and using three electrode systems, optimum square wave (SW) stripping results were attained to 1.0-9.0 ug/L with 8 points. Statistic RSD precision was of 6.02 % with n=15 in 0.1 mg/L glucose. After a total of 200 second accumulation times, analytical detection limit of 0.8 ug/L was obtained. This developed technique was applied to urine samples from diabetic patients urine for fluid analysis, it was determined that the sensor can be used with a diagnostics in the ex vivo of live cells and non treated biological fluid.

Keywords: glucose, fluorine, nafion, voltammetry, diabetic

1. Introduction

In vivo or food systems, highly concentrated glucose was associated with a number of biological diseases, such as ischemic heart disease, cardiovascular events [1], diabetes mellitus, elevated blood pressure [2], end stage renal disease [3] and central nervous disease. Since metabolic assays are particularly important in live cell and in vivo blood, diabetic patients are required to undergo daily glucose analysis in a vein. Numerous needle suctions are painful, which is why urine assay is a better substitutive effect in blood assay, while urine analysis require a very low analytical detection limit (DL). Recently, various diagnostic methods have been developed, which include capillary electrophoresis with precolumn derivatization and UV detection (DL of 3.82-4.14 mg/L) [4], high performance liquid chromatography with refractive index method (DL:0.13 mg/ml,) [5] and others. The same technique, which attained high detection range, also demands complicated temperature control, liquid compressor, separation techniques and other detection systems. On the other hand, voltammetric methods, which have already been developed, are simple and compact. These methods arebienzymatic biosensor (DL: 0.07

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mM) [6], photopolymeric membrane for amperometric glucose biosensor (linear range of 0.1±5 mM)[7], electrochemical synthesized poly film method (linear range of 0.5-18.0 mM)[8] and others. They have high detection limits but are not useable in in-vivo fluid. For this reason, it was necessary to look for a more sensitive method using fluorine doped [9] on a nafion composed [10] multi-walled carbon nanotubes [11] paste[12] electrode (FNCE). This new type of sensor was optimized using cyclic and square-wave stripping voltammetry and was used for diagnostic analysis that have been found useful in stripping voltammetry. The anodic voltammograms appeared to be very sensitive within the nano-level working ranges. There are modifications that can be attained to lower detection limit compared to that of the previous method. These developed techniques can be applied to human urine for ex vivo diagnosis.

2. Experimental procedure

2.1. Instruments, sensors, chemicals and procedures

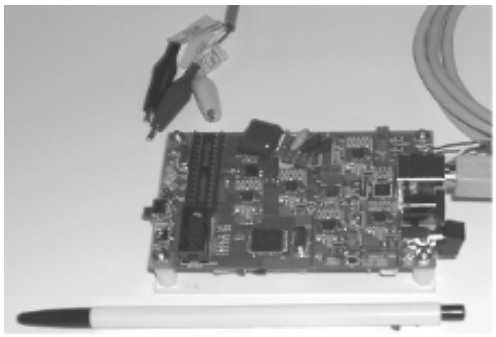


Fig. 1. The Bioelectronics-2 second version, simple and small with three electrode systems for trace biological analysis.

A voltammetric circuit was carried out using the new Bioelectronics-2 system. The second version was pioneered by the authors' institution. Figure 1 shows the tiny, compact circuit, which is smaller than a regular pen.

The 3"×2"×1" compact sized second version is a computerized handheld voltammetric system with a 3.0 V potential range, a 2 mA current range, and a 10pA measuring current. It uses a rechargeable battery with USB power source and has a USB port data telecommunication interface with a PC. The instrument's size is similar to that of a typical cellular phone and can be used for bioassay, microorganisms poisoning detection and sensor techniques for individual and laboratory applications. It can apply for Bluetooth personal systems. The common type graphite pencil carbon (PE) reference and counter was prepared with a 0.5x10 mm diameter Hipolymer HB. H and 3H grade pencil leads (Pentel, Japan) were used. The FCNE working electrode was hand-made from multi-walled carbon nanotubes, which has catalytic CVD, outside diameter of 15-40 nm, 30-50 nm long and was produced by Nanotech Co., Ltd., Choongnam, South Korea 330-816. They were purified overnight prior to use in a 2-M nitric-acid and triple-distilled water, which was prepared by mixing 60 % carbon nanotube and 20 % nafion and 20 % HF solution (standard). The mixed paste was inserted into a 2-mm-diameter × 50-mm-long capillary (inside paraffin coating) glass using a copper wire connected to the electric system. Glucose standard solution (1000 mg/L) was obtained from Merck. The 1000 ug/L standard solution was prepared diluting 0.1 mL of 1000 mg/L into 100 mL pure water. A 1000 ng/L standard solution was also prepared using the same method. All the chemicals that were used were of analytical purity. Highly purified water was prepared using a three-time distillation process with 18 MΩ/cm of Milli Q Ultra Pure Water System (Millipore, Bedford, USA). The urine sample was obtained from our institute center and the National Blood Research Center. The

FCNE electrode was stabilized in a 10 mL 0.1M NH4H2PO4 solution using a 10-cycle scan in blank electrolyte conditions with a 1.0 V initial potential, 1.0 V switching potential, and 0.5 V/s scan rate, so that the -HF-nafion-c- structure can be immobilized and then used in the optimization. All experiments were performed at room temperature, without removing the oxygen.

3. Results and discussion

3.1. Comparing PE, FCNE and stability

First, reaction potential was examined by cyclic scan. Under this condition, electrode sensitivity and sensor stability were examined. Figure 2A shows the glucose variation using common type PE from 0 to 140 mg/L spike. Peak potential appeared at 0.35 V. Current height increased from 0.67x10-6 to 10.3 x10-6A. Also, linear equation was obtained at y=0.0685x-0.0571, R2=0.9865, which are wide analytical ranges and can be used in high ranges. However, better sensitive low ranges were examined. Figure 2 (B) shows SW

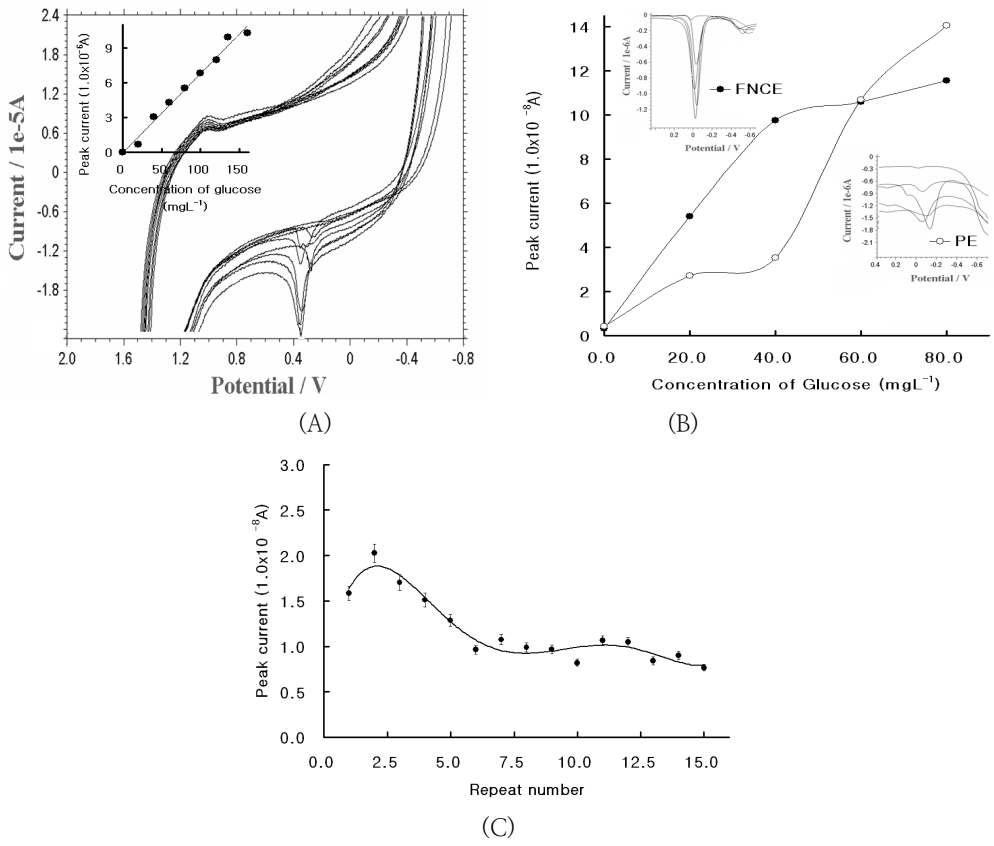
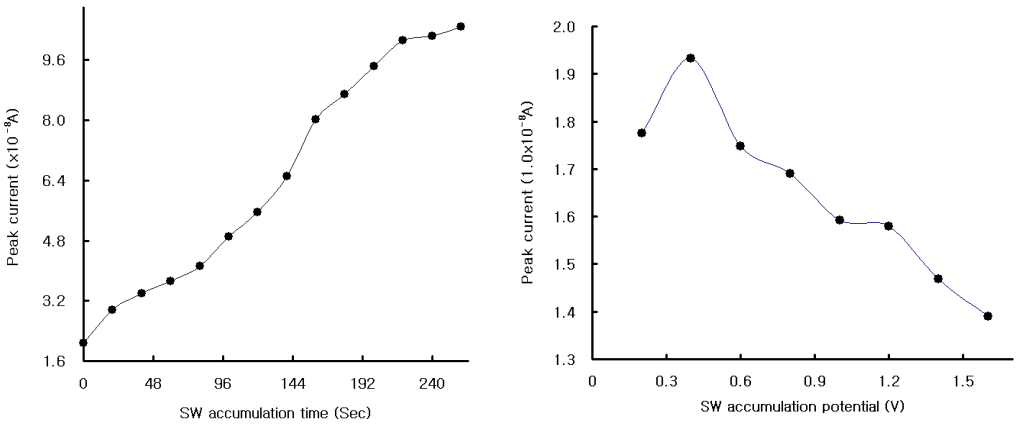


Fig. 2. (A): CV effects for glucose variation with 0, 20, 40, 60, 80, 100, 120, 140, and 160 mg/L spike using PE; Figure 1(B): Electrode comparison for FNCE and PE with SW accumulation time of 30 sec, 0, 20, 40, 60 and 80 mg/L glucose variations, anodic stripping in 0.1M $NH_4H_2PO_4$ electrolyte solution; Figure 1 (C); 15th repetition using 0.1 mg/L addition with optimum condition

stripping voltammetry by the electrode comparison with common PE type and specially prepared FCNE in the same electrolyte conditions. Glucose varied from 0 to 80 mg/L, with a peak height at 0.31-11.55x10-8A (FCNE) and 0.41-14.05x10-8A (PE). However, FCNE peak width was very sharp, while PE is broad and not sensitive. Here, FCNE was applicable for low range assays. In this condition, stable electrode stable examined by replications for the 15th determination. Figure 2(C) shows the results. The initial 4th peak was oscillated and linear, which a peak current from 1.58x10-8A to 0.74x10-8A. Standard deviation yielded good results for 0.387, which is usable in SW stripping voltammetry. SW optimum conditions were examined.

3.2. Evaluating stripping voltammetric optimizations

Using FNCE, variations of the SW accumulation time were studied by fixing the 20 mg/L spike. The peak currents that were obtained are shown in Figure 3A. On the FNCE oxidation, peak current was shown to have increased, was very sensitive and had linear rising, then steadied. Subsequently, a 60-second accumulation time was chosen as the optimum SW stripping. At this point, SW accumulation potentials were searched by the FNCE. The obtained signal is shown in Figure 3B, which was within the positive range of 0.0 to 1.6 V. Peak currents appeared to have a very narrow half-width at 0.5 V, which then decreased quickly. Thus, the optimum accumulation potential of 0.5 V FNCE was chosen for SW. Under these parameters, optimum SW conditions were obtained for 0.1 V amplitude, 15 Hz frequency, 4 mV increment potential and 4.06 pH. The oxidation peak was appeared to be very sharp. Herein, the analytical interference effects for the metal and organic ions were examined at 0.5 mg/L constant using tenfold spiking. Here, the effect was calibrated using the standard addition method. Analytically working ranges and diagnostic investigations were then



(A) (B)

Fig. 3. (A): Variations of the SW accumulation time range for 0.0, to 260 seconds; (B): SW accumulation potential ranges from 0.2 to 1.6 V, additions of 20 mg/L glucose in the 0.1 M $NH_4H_2PO_4$ electrolyte. Other parameters were used for the following optimum conditions: pH 4.06, 0.1 V SW amplitude, 15 Hz SW frequency, 4 mV increment potential, -2.0 V final potential, and 120-seconds accumulation time

searched.

3.3. Analytical working ranges, statistics and urine test

Using the final parameters, analytical working ranges were examined to milli- and micro-spike with FCNE in the SW condition. Raw voltammograms and working equations are shown in Figure 4 (A) for 0.02-0.14 variations. Here, the peak current was obtained for 9.27-20.82±0.005x10-8A at -0.10 V oxidation potential. Peak potential was negative, as it shifted from 0.0 V to -0.1 V; slop sensitive was △x/△y=59.733; precision was at 0.9733=R2, which was only used for 120 seconds, and can be applied for a diagnostic assay. However, more advanced detection ranges were examined in 200 seconds using the same cell systems. Figure 4(B) shows the results for micro working ranges from 1 to

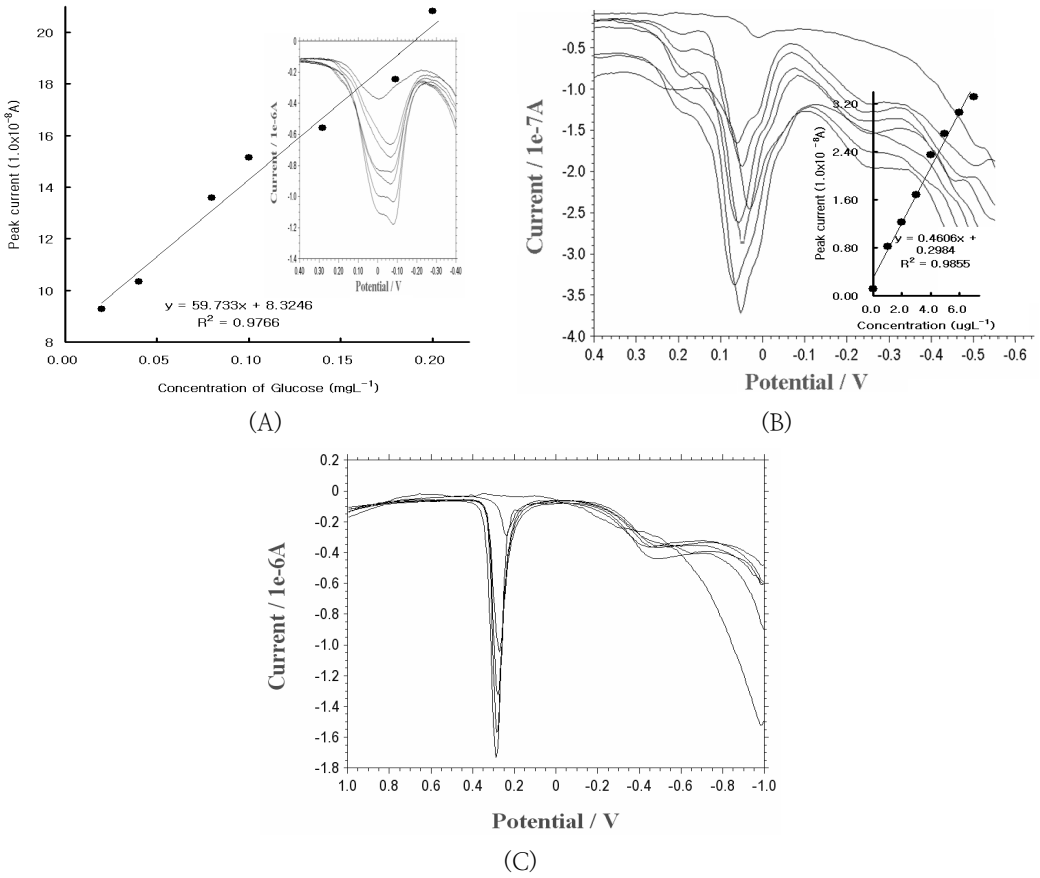


Fig. 4. (A): Using optimum conditions, linear working ranges of 0.02, 0.04 0.06 0.08 0.10, 0.12 and 0.14 mg/L spikes; (B): FCNE working ranges of 1, 2, 3, 5, 6, 7 and 8 ug/L spike in a 0.1M $NH_4H_2PO_4$ electrolyte solution with a pH of 3.06, an SW amplitude of 0.1 V, an SW frequency of 15 Hz, an increment potential of 4 mV, an accumulation potential of 0.5 V, and an accumulation time of 120 seconds; (C) Patient urine test, the first curve represents the blank and 0.05 mL urine and 0.01, 0,02, 0.03, 0.04 ml standard glucose spike with the SW parameter used for optimum conditions

8 ug/L additions. Here, the peak potential appeared at 0.0 V and did not shift to any directions, where peak current varied from 0.11 to 3.14±0.006x10-8A, slop sensitive of △x/ △y=0.46, precision at 0.98=R2, which may be useable for any human fluid, Under such conditions, statistic precision was examined with the replicated 15th determination of the 1.0 mg/L standard, from which 0.970 % of RSD appeared. These results are highly reproducible and can be suitable for in vivo or ex vivo diagnosis. Moreover, the developed results were examined for the analytical detection limits using KSb/m (k=3, n=15, m=△x/△y) and obtained 0.8 ug/L (S/N=3) SW, showing that they are better sensitive than the previous methods. Herein analytical application was performed on the urine of diabetic patients and healthy individuals. Figure 4(C) shows application for the urine tests from diabetic patients. The first curve is electrolyte blank and urine, standard spike, where the standard addition method was obtained for 6.3-7.5 mg/L. However, those from healthy individuals had 2.0-2.8 mg/L. The methods developed herein can be applied to human fluid in ex vivo, and in the diagnosis of live organs.

4. Conclusion

The developed FCNE combination sensor with hand-held analyzer described herein is for the assay of glucose. The optimized parameters of 0.1 V accumulation potential, 0.1 V amplitude, 15 Hz frequency, 4 mV increment potential, 4.06 pH electrolyte, and 120 second accumulation time were obtained, where analytical results attained 1 to 8 ug/L working range, a lower detection limit of 0.6 ug/L and the precision of 0.98 R2, which diagnostic parameter was applied on urine tests from diabetic patients and healthy individuals. It can also be applied in other fields requiring in in vivo or ex vitro diagnosis for human body systems.

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